

ATTACHMENT 2

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Figure 5 illustrates an investigational device (by Medtronic, Inc. of Minneapolis, MN - schematic of Model 8506), which can be implanted subcutaneously on the cranium, and provides an access port through which therapeutic agents may be delivered to the brain.

5 Figure 6 illustrates the relation of various neurodegenerative diseases described herein, and the location of treatment with small interfering RNA vectors directed to their intended targeted gene product.

Figure 7 is a schematic side view depiction of a marker tip at the distal end of the catheter used in implementing the method of the invention.

10 **DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS**

The present invention solves two problems in the prior art at the same time: (1) the problem of how to treat neurodegenerative diseases caused by the production in neurons of a protein that has pathogenic properties and (2) the problem of delivery of therapeutic small interfering RNA to affected neurons.

15 In order to better understand the present invention, a list of terms and the scope of understanding of those terms is provided below.

Terminology

20 By "alpha-synuclein, BACE1 (including variants thereof, e.g. variants A, B, C, and D), huntingtin, ataxin-1, ataxin-3, and/or atrophin-1 proteins" is meant, a protein or a mutant protein derivative thereof, comprising the amino-acid sequence expressed and/or encoded by alpha-synuclein (Parkinson's disease), and beta-site APP-cleaving enzyme (BACE1 (including variants thereof, e.g. variants A, B, C, and D)) (Alzheimer's disease), huntingtin (Huntington's disease), and ataxin-1 (Spinocerebellar Ataxia Type 1), ataxin-3 (Spinocerebellar Ataxia Type 3 or Machado-Joseph's Disease), and/or dentatorubral-pallidoluysian atrophy (DRPLA) genes and/or the human genomic DNA respectively.

25 As used herein "cell" is used in its usual biological sense, and does not refer to an entire multicellular organism. The cell may be present in an organism which may be a human but is preferably of mammalian origin, e.g., such as humans, cows, sheep, apes, monkeys, swine, dogs, cats, and the like. However, several steps of producing small

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By "small interfering RNA" is meant a nucleic acid molecule which has complementarity in a substrate binding region to a specified gene target, and which acts to specifically guide enzymes in the host cell to cleave the target RNA. That is, the small interfering RNA by virtue of the specificity of its sequence and its homology to the RNA target is able to cause cleavage of the RNA strand and thereby inactivate a target RNA molecule because it is no longer able to be transcribed. These complementary regions allow sufficient hybridization of the small interfering RNA to the target RNA and thus permit cleavage. One hundred percent complementarity often necessary for biological activity and therefore is preferred, but complementarity as low as 90% may also be useful in this invention. The specific small interfering RNA described in the present application are not meant to be limiting and those skilled in the art will recognize that all that is important in a small interfering RNA of this invention is that it have a specific substrate binding site which is complementary to one or more of the target nucleic acid regions.

Small interfering RNAs are double stranded RNA agents that have complementarity to (i.e., able to base-pair with) a portion of the target RNA (generally messenger RNA). Generally, such complementarity is 100%, but can be less if desired, such as 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%. For example, 19 bases out of 21 bases may be base-paired. In some instances, where selection between various allelic variants is desired, 100% complementary to the target gene is required in order to effectively discern the target sequence from the other allelic sequence. When selecting between allelic targets, choice of length is also an important factor because it is the other factor involved in the percent complementary and the ability to differentiate between allelic differences.

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The small interfering RNA sequence needs to be of sufficient length to bring the small interfering RNA and target RNA together through complementary base-pairing interactions. The small interfering RNA of the invention may be of varying lengths. The length of the small interfering RNA is preferably greater than or equal to ten nucleotides and of sufficient length to stably interact with the target RNA; specifically 15-30 nucleotides; more specifically any integer between 15 and 30 nucleotides, such as 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, and 30. By "sufficient length" is meant

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these and other devices and systems may be suitable for delivery of small interfering RNA vectors for the treatment of neurodegenerative diseases in accordance with the present invention.

In one preferred embodiment, the method further comprises the steps of 5 implanting a pump outside the brain, the pump coupled to a proximal end of the catheter, and operating the pump to deliver the predetermined dosage of the at least one small interfering RNA or small interfering RNA vector through the discharge portion of the catheter. A further embodiment comprises the further step of periodically refreshing a supply of the at least one small interfering RNA or small interfering RNA vector to the 10 pump outside said brain.

Thus, the present invention includes the delivery of small interfering RNA vectors using an implantable pump and catheter, like that taught in U.S. Patent No. 5,735,814 and 6,042 579, and further using a sensor as part of the infusion system to regulate the amount of small interfering RNA vectors delivered to the brain, like that taught in U.S. 15 Patent No. 5,814,014. Other devices and systems can be used in accordance with the method of the present invention, for example, the devices and systems disclosed in U.S. Serial Nos. 09/872,698 (filed June 1, 2001) and 09/864,646 (filed May 23, 2001), which are incorporated herein by reference.

20 It is preferred to place some means for locating distal end 14 during the access and location process. This is preferably done by applying a marker 46, as shown in FIG. 7, to distal end 14 which is detected during the access and location process. If access and location is accomplished using some form of x-ray radiation, marker 46 is preferably radiopaque. Radiopaque marker 46 renders at least a portion of distal tip 14 opaque to x-rays, enabling the tip 34 to be observed via fluoroscopy or via x-ray during access and 25 location of catheter 10.

30 In a preferred embodiment, radiopaque marker 46 comprises tantalum powder dispersed in a matrix composed of a biocompatible adhesive, such as those discussed above. Ordinarily, radiopaque marker 46 will be premolded prior to insertion into the lumen 38. After radiopaque marker 46 has been inserted into the lumen 38, a thin coating of the same biocompatible adhesive is preferably applied to the exterior of the hemispherical portion 48. Other materials may also be suitable for radiopaque marker 46.

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such as barium or platinum materials.

Alternately, the radiographic marker 46 may be chosen of a material that has sufficient radiodensity for visualization during radiologic procedures, but in powdered form that is dispersed in the catheter tip 34 at the time the catheter tip 34 is molded.

5 Alternatively, marker 46 may be composed of a material that is compatible to nuclear magnetic resonance imaging (MRI) to enable the tip 34 to be detected during an MRI scan. Preferred material for such a marker 46 is platinum, though barium, tantalum, and similar materials are also suitable. Regardless of whether radiography or MRI is being utilized, the goal of providing a radiographic marker 46 is to enable the operator to
10 accurately detect the precise location of the tip 34 to facilitate placement and later verification of the integrity and position of distal end 14 of catheter 10.

To summarize, the present invention provides methods to deliver small interfering RNA vectors to the human central nervous system, and thus treat neurodegenerative diseases by reducing the production of a pathogenic protein within neurons.

15 The present invention is directed for use as a treatment for neurodegenerative disorders and/or diseases, comprising Alzheimer's disease, Parkinson's disease, Huntington's disease, Spinocerebellar type 1, type 2, and type 3, and/or any neurodegenerative disease caused or aggravated by the production of a pathogenic protein, or any other neurodegenerative disease caused by the gain of a new, pathogenic function by a mutant protein.

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promoter primer, and filled-in with Klenow fragment to generate a full-length DNA template for transcription into RNA. Two *in vitro* transcribed RNAs (one *anti* sense to the other) were generated by *in vitro* transcription reactions then hybridized to each other to make double-stranded RNA. The double-stranded RNA product was 5 treated with DNase (to remove the DNA transcription templates) and RNase (to polish the ends of the double-stranded RNA), and column purified to provide the three siRNAs that were delivered and tested in cells.

Example 2: Delivery of a small interfering RNA targeting human ataxin1 mRNA.

10 The constructed siRNA molecules 1-3 described in Example 1 were transfected into HEK293 cells. The RNA produced by the transfected cells was harvested and assayed to measure the amount of human ataxin1 mRNA.

15 Figure 1 shows the results of a quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) assay for the amount of ataxin1 messenger RNA (mRNA) per microgram of total RNA from cultures of HEK 293H cells. Four cell populations were assayed. The first were 293H cells that had been transiently transfected with siRNA against GAPDH, a "housekeeping gene" with no known relationship to ataxin1 mRNA expression. (The siRNA against GAPDH was supplied as a standard control by Ambion, Inc., in their commercially-available kit for making and testing siRNA). The second 20 were 293H cells that had been transiently transfected with siRNA against ataxin1 mRNA at location 1671 in the ataxin1 mRNA sequence. The third were 293H cells transiently transfected with a plasmid containing a ribozyme against ataxin1 mRNA (which cleaves ataxin1 mRNA at position 1364 in the ataxin1 mRNA sequence). The fourth were 293H cells transiently transfected with siRNA against ataxin1 mRNA at location 0945. All cell 25 populations were harvested concurrently for total cellular RNA, at a time point 48 hours after transfection.

30 On the gels pictured, the amplified DNA products of the RT-PCR reaction were separated by molecular size, using gel electrophoresis, and are visible as bands of varying intensity. Each cell population described was assayed using a series of parallel reactions, shown as a set of lanes at the top or bottom of each gel. Each set of lanes contains two bands per lane. The top band is the DNA product amplified from a known quantity of

ATTACHMENT 3

**Replacement Sheets for
Pages 9, 14, 29-35 of the
Specification and Figure 7**